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Paralytic shellfish poisoning surveillance in California using the Jellett Rapid PSP test

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Abstract

In a previous publication, we reported equivalent presence/absence results for paralytic shellfish poisoning (PSP) detection using three in vitro assays and the mouse bioassay (MBA). In January of 2004, in response to the summary of actions from the 2003 Interstate Shellfish Sanitation Conference, the U.S. Food and Drug Administration concurred that the Jellett Rapid PSP test (JRPT) may be used for screening acidified shellfish tissues for the saxitoxins.

A parallel study of the JRPT and the MBA was conducted from January 21 to April 13, 2004. Thereafter, the JRPT was implemented as a PSP screen for the remainder of 2004. A negative JRPT test represented a final result. When the JRPT was positive or indeterminate, the MBA was conducted.

From January 21 to December 23, 2004 a total of 910 JRPT were completed; the testing yielded 478 negative, 147 positive, 259 false positive, 20 indeterminate and 6 invalid results. Animal usage was decreased and analyst time was conserved when a negative screen was obtained. The study confirmed the JRPT could be used for PSP surveillance in California on a year-round basis without a negative impact on public health.

Keywords: Saxitoxin; PSP; In vitro methods

1. Introduction

California has a long-standing history of monitoring shellfish for marine biotoxins. The human syndrome associated with the consumption of saxitoxin containing shellfish is known as paralytic shellfish poisoning (PSP). This work is conducted in the state's public health laboratory. California's PSP workload in a typical year varies from 800 to 1000 samples and is typical for a lab that processes commercial and volunteer samples submitted in support of a pre-harvest surveillance program. From November to April the incidence of phytoplankton blooms is low compared to the months of May–October. This historical pattern led to the statewide use

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of PSP buoys, which alert the public to the danger of eating shellfish taken from the posted areas.

Phytoplankton blooms occur at unpredictable times throughout the year. The spikes in PSP concentration measured from November to April are sporadic; toxicity levels rarely exceed the closure limit. The number of samples arriving in the lab varies from one to eight samples per day with the heaviest workload falling on Wednesday and Thursday. Volunteer and commercial shellfish submitters usually wash, shuck, drain, freeze the samples and ship overnight to the lab for a morning arrival. The extraction process is completed by noon and final reports are available by 5 p.m. The workload from May to October parallels the greater frequency of phytoplankton blooms. Samples from commercial growing areas predominate when blooms occur close to the harvest sites. This triggers increased sampling and submission of shellfish on a daily basis, thus increasing the workload.

Following the 2003 Interstate Shellfish Sanitation Conference (ISSC), a summary of committee actions was posted on the ISSC website (http:www.issc.org). In January of 2004, the U.S. Food and Drug Administration (US-FDA) sent a letter to the ISSC on proposal 03-116 concurring that the Jellett Rapid PSP test (Jellett Rapid Testing Ltd., NS, Canada) may be used for screening shellfish for the PSP toxins.

Shortly after the issuance of the letter from US-FDA, our laboratory initiated a parallel study with the Jellett Rapid PSP test (JRPT). The initial study included the evaluation of 232 AOAC/APHL extracted tissues of mussels and oysters submitted for routine PSP surveillance from January 21 to April 13.

After review of the parallel study data, the JRPT was implemented as a screening tool for the remainder of 2004; the mouse bioassay (MBA) served as the confirmatory test. The goal was to gather sufficient data to aid decision making for seasonal or year-round PSP screening.

2. Materials and methods

The samples were representative of shellfish along the entire length of California coastline and constituted mussels and oysters. Raw, shucked, drained and frozen shellfish meats were submitted by coastal volunteer collectors and by commercial growers. These arrived at the lab frozen or as thawed, cold meats packed in polypropylene blender jars surrounded by refrigerant packs. An occasional sample arrived as shellstock.

Raw shellfish meats were extracted with the AOAC/APHL procedure (APHA, 1970) that yields a 1:2 dilution of the original tissue. The MBA was conducted as specified in the AOAC official method (APHA, 1970; Hungerford, 1995). Each 1:2 acidified extract was diluted in the manufacturer's diluent at the suggested 1:5 ratio and applied to one cassette. JRPTs were recorded as positive or negative or indeterminate at the designated 20 min time limit based upon the color chart provided with each kit. Two analysts reviewed each kit.

The State's Laboratory Central Services Branch (LCSB) provided the Swiss Webster mice. All aspects of the MBA were followed, including the injection of a minimum of three mice per sample; a weekly ''conversion factor'' (CF) ensured the mouse colony was stable in its response to the saxitoxin (STX) standard. Dr. Sherwood Hall, US-FDA National Seafood Laboratory, Washington, DC provided the STX standard. Data were recorded as μg/100 g. Samples were diluted, as needed, in 3 mM HCl to achieve an endpoint in the MBA. Notations were made on the MBA when animals were sick and lived.

The initial 232 samples were screened in a parallel study design. The first samples were tested on January 21 and the last on April 13. Each extract was analyzed with the JRPT and with the MBA. Samples were analyzed one time only. Data were entered into a spreadsheet by month of testing, sorted and tabulated. Results were categorized as true negative, true positive, false positive or indeterminate. The mouse bioassay represented the true value for the purposes of this parallel study.

Starting April 14, 2004 we screened acidified extracts with the JRPT and conducted a MBA only on those samples that were JRPT positive or indeterminate. We did not screen every sample with the JRPT. Once an individual collection site or a nearby sampling area yielded two consecutive MBA confirmed positives, we ceased to use the JRPT until a negative MBA was observed for that site. In addition, there were periods of time, due to fiscal or purchasing restraints, when we lacked a stock of the kits. Therefore, the data presented in this study do not represent our entire workload.

3. Results

The data from the parallel study of the JRPT and the mouse bioassay established confidence in the in vitro test. There were no false negatives. Out of the 232 extracts screened initially, there was a 29% false positive incidence, wherein the JRPT was positive and the mouse bioassay was negative. However, lab notes identified that many of the animals were sick with PSP symptoms although they did not die. One extract yielded an indeterminate result by JRPT and was repeated, yielding a negative test by both methods. The parallel study was conducted from January 21 to April 13, California's low PSP season. Oysters comprised 27% of the samples and mussels the remaining 73%. A total of 233 tests were completed on 232 extracts.

Data gathered from April 14 to December 23, 2004 covered the high and low PSP seasons in California. Mussels represented commercial growing areas and recreational areas, whereas oysters were submitted from commercial growing areas only. A total of 909 shellfish were extracted and the dataset represents 910 tests, which includes the one repeat test.

Using the JRPT, there were 478 negative, 147 positive, 259 false positive and 20 indeterminate test results. Six tests were invalidated due to a laboratory error discovered after the extraction and testing process. No repeat JRPT tests were conducted from April 14 to December 23; the mouse bioassay was used as the definitive test in these cases.

The percentage of data by month and result are depicted in Fig. 1. Greater than 60% of tests conducted in January, February, March and May yielded negative results. More PSP positives were detected during the months of June, July and August. April and August yielded greater than 40% false positive screens and March, September and October were above 30%.

Collated data for PSP low, high and combined seasons is presented in Table 1. For all the months, 52.5% of tests were negative and 16% were positive.

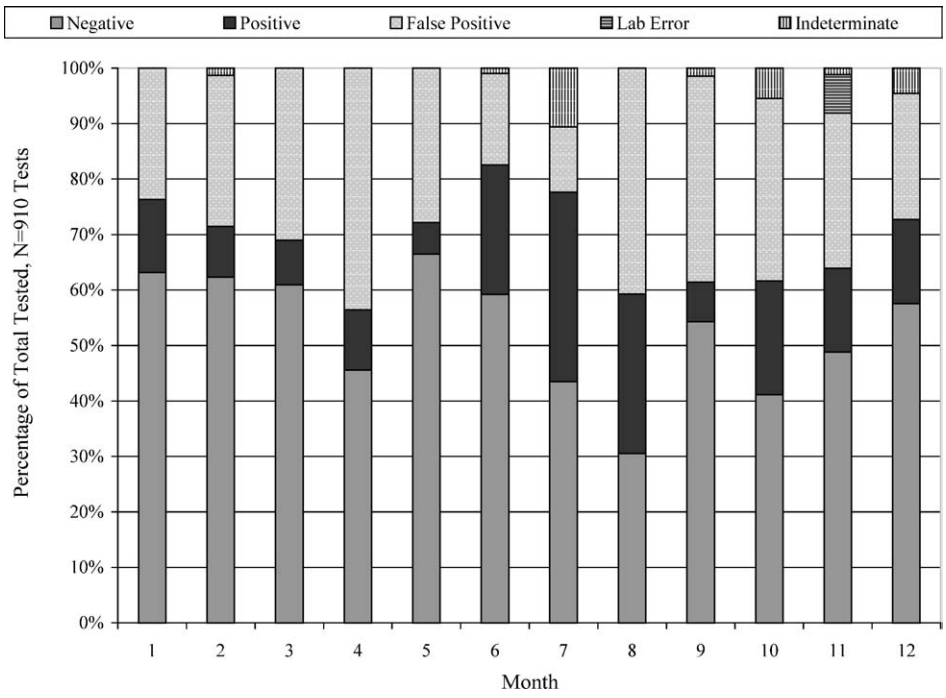

Fig. 1. Data by month and result for shellfish extracts screened using the Jellett Rapid PSP test from January 21 to December 23, 2004. Negative, Jellett Rapid PSP test negative. Positive, Jellett Rapid PSP test positive. False positive, Jellett Rapid PSP test positive and mouse bioassay negative. Lab error, test results were invalidated due to a laboratory error. Indeterminate, test results were invalidated due to one of two conditions observed during the test: (1) test substance failed to migrate or (2) the intensity of the control line "C" was 25% less than the demonstration unit "C" line.

Table 1
Summary of 910 tests conducted with the Jellett Rapid PSP test (JRPT) from January 21 to December 23, 2004 using acidified mussel and oyster extracts

Result	Number of JRPT tests		
	Low season[a]	High season[b]	Combined seasons[c]
Negative	251	227	478
Positive	53	94	147
False positive[d]	140	119	259
Lab error[e]	6	0	6
Indeterminate[f]	5	15	20
Total	455	455	910

[a] Low season, JRPT tests conducted from November 1 to April 30.
[b] High season, JRPT tests conducted from May 1 to October 31.
[c] Combined seasons, JRPT tests conducted from January 21, 2004 to December 23, 2004.
[d] False positive, the term used when the JRPT was positive and the mouse bioassay was negative.
[e] Lab error, invalidation of a test due to an error by lab staff.
[f] Indeterminate, when either the extract did not migrate across the JRPT strip or the intensity of the control line "C" was 25% less than the manufacturer's demonstration unit "C" line.

The JRPT false positive rate varied from an average of 30.8% during low season to an average of 26.2% during high season. The percentage of false positives based upon 910 tests was 28.5%, whereas the parallel study false positive rate was 29% for 233 tests. There was no apparent difference in performance of the JRPT with oyster versus mussel extracts.

4. Discussion

An evaluation of California's historical PSP data showed that in a typical year most numerical data fell below or equal to 40 μg per 100 g of shellfish meat. We previously projected that our lab might reduce animal use by 35–50% during November–April if an in vitro screen was utilized to separate the PSP negative samples from ones needing follow-up mouse bioassays.

A test that was positive in the JRPT and negative in the MBA was categorized as a "false positive". The percentage of false positives observed in the first 233 tests was consistent through the remaining months of screening. For the lab, this meant we would not achieve the optimum savings in animals projected by our historical MBA data.

Previously, in the testing of twice frozen shellfish with the predecessor MIST Alert™ test (Jellett Biotek Ltd., NS, Canada), we observed a low false positive rate of 8% (Inami et al., 2004); this raised the expectation that similar results would be obtained with the JRPT, since it was based on the same technology and the same lower limit of detection. However, the extracts selected for that study were chosen on the basis of clean animal data. Extracts wherein one or two animals died or extracts where animals were sick but did not die were not used. We did not have a chromatography instrument to investigate the false positives. Jellett et al. (2002) reported a false positive rate of 14% in their study of 2100 extracts with the MIST Alert test. They also provided an explanation on toxicity levels in the false positive samples examined by HPLC.

A substantial number of extracts were positive in the JRPT and negative by the MBA. In many of these cases, the animals were sick but no animals died. In other cases, one or two animals died, but the median mouse was alive resulting in a MBA negative outcome. If one considers the 36 μg/100 ml limit of detection for the MBA (Hall, 1991) and the ±20% variability of the MBA (Sommer and Meyer, 1937), one could expect to see a possible 20% discrepancy in outcomes between the MBA and the JRPT on samples near the limit of detection.

Even with the high false positive rate, there was a projected 30% animal reduction if the JRPT was implemented for screening during low PSP season. At the end of April, the State veterinarian was asked to reduce mouse production for the PSP program based upon the January–April parallel study.

The data compiled for the low and high PSP seasons in California showed the JRPT was beneficial for year-round screening. More JRPT positives were observed during high PSP season as would be expected. April and July had the highest ratio of false positive tests. The high number of false positives when the two assays were compared had no consequence on overall public health protection, since the mouse bioassay was used for confirmation.

The decision point for stopping the JRPT screen or restarting it for shellfish collected from any given sampling point or area was not simple. Over the course of the year, we realized there were many factors that needed consideration. These included the following:

(1) month, (2) commercial or recreational beds, (3) frequency of sample collection, (4) magnitude of a MBA value, (5) phytoplankton sightings, (6) weather predictions, (7) input from our regulatory colleagues and (8) complexity of the sampling site or area. Our biggest challenge came with the monitoring of a very large commercial growing area with seven harvest beds and one sentinel site situated in an estero.

Our experience during low and high PSP seasons led to the establishment of these basic guidelines for JRPT screening. One, a single JRPT positive with a confirmed MBA above 80 μg/100 g from a single shellfish collection point is an indicator that a bloom is established and levels may go higher. Therefore, omit the JRPT screen and go directly to the MBA on future samples from that site. Two, when consecutive shellfish samples from the same site are JRPT positive with MBA values between 40 and 80 μg/100 g, environmental conditions are favorable for the phytoplankton to persist. On future samples from that single site, skip the screen. Three, when one shellfish sample is submitted and the JRPT and MBA are positive, any subsequent shellfish submitted within a 7-day period should be tested with the MBA and not the screen. Four, when three separate sampling points from a complex growing area are JRPT positive and confirmed MBA positive, then use the MBA on remaining sampling points associated with that area. This guidance is for economic reasons. One has to use judgment in deciding when to reinitiate JRPT screening for any sampling point or area.

Each laboratory that adopts the JRPT for screening shellfish for PSP will need to develop their own guidelines for use. Our guidance was developed after we finished a year of screening with the JRPT, another lab might have a completely different experience. In the coming months, judgments will be made regarding economics, animal use and screening with the JRPT.

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